

## CLAIMS

## WE CLAIM:

1. A human pluripotent cell culture, wherein the cells of the culture do not express  
5 SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
2. The cell culture of Claim 1, wherein the cell culture was dissociated to an essentially single cell culture.
3. The cell culture of Claim 2, wherein a majority of the cells have an abnormal  
10 karyotype.
4. The cell culture of Claim 3, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
5. The cell culture of Claim 4, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
- 15 6. The cell culture of Claim 5, wherein the autosomal chromosome is chromosome 12 or 17.
7. The cell culture of Claim 3, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
8. The cell culture of Claim 7, wherein at least one of the more than one autosomal  
20 chromosomes is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
9. The cell culture of Claim 8, wherein at least one of the more than one autosomal chromosomes is selected from the group consisting of chromosome 12 or 17.
10. The cell culture of Claim 3, wherein the abnormal karyotype comprises an  
25 additional sex chromosome.
11. The cell culture of Claim 10, wherein the karyotype comprises two X chromosomes and one Y chromosome.
12. The cell culture of Claim 3, wherein the cells are stable in culture for greater than approximately 10 passages.
- 30 13. A method of culturing a human pluripotent cell comprising,
  - a) selecting a human pluripotent cell using an anti-SSEA4 antibody;  
and
  - b) maintaining a culture of the cell by passaging the cell using a protease treatment, wherein the cells of the culture do not express

SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.

14. The method of Claim 13, wherein the protease treatment comprises the sequential use of Collagenase and trypsin.
- 5 15. The method of Claim 13, wherein the cell is maintained by using a protease treatment for at least 13 passages.
16. The method of Claim 13, wherein a majority of the cells of the culture have an abnormal karyotype.
17. The method of Claim 16, wherein the abnormal karyotype comprises a trisomy  
10 of at least one autosomal chromosome.
18. The method of Claim 17, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
19. The method of Claim 16, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
- 15 20. The method of Claim 19, wherein at least one of the more than one autosomal chromosomes is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
21. The method of Claim 16, wherein the abnormal karyotype comprises an additional sex chromosome.
- 20 22. The method of Claim 21, wherein the karyotype comprises two X chromosomes and one Y chromosome.
23. The method of Claim 14, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.
- 25 24. A method of providing a human cell culture enriched in neural cells, comprising forming an embryoid body comprising the human pluripotent cell of Claim 13.
25. The method of Claim 24, wherein the embryoid body is formed by culturing the cell with an essentially serum free medium.
26. The method of Claim 25, wherein the essentially serum free medium is a  
30 MEDII conditioned medium.
27. The method of Claim 26, wherein the MEDII conditioned medium is a Hep G2 conditioned medium.
28. The method of Claim 26, wherein the MEDII conditioned medium comprises one or more proline residues or a polypeptide containing proline residues.

29. The method of Claim 28, wherein the MEDII conditioned medium comprises proline at a concentration of approximately 50  $\mu$ M.
30. The method of Claim 24, wherein the embryoid body is formed by culturing the cell with a minimal medium.
- 5 31. The method of Claim 30, wherein the minimal medium is essentially proline free.
32. The method of Claim 30, wherein the minimal medium comprises one or more proline residues, or a polypeptide containing proline residues.
33. The method of Claim 32, wherein the minimal medium comprises proline at a concentration from approximately 50  $\mu$ M to approximately 250  $\mu$ M.
- 10 34. The method of Claim 30, wherein the minimal medium is essentially FGF free.
35. The method of Claim 30, wherein the minimal medium is essentially MEDII free.
36. The method of Claim 13, wherein the human pluripotent cell is selected from the group consisting of a human embryonic stem cell, a human inner cell mass (ICM)/epiblast cell, a human primitive ectoderm cell, and a human primordial germ cell.
- 15 37. The method of Claim 13, wherein the human pluripotent cell is a human embryonic stem cell.
- 20 38. A human pluripotent cell produced by the method of Claim 13.
39. A human cell culture enriched in neural cells, produced by any the method of any one of Claims 24-37.
40. The human cell culture of Claim 39, wherein greater than approximately 80% of the human cell culture comprises neural cells.
- 25 41. The human cell culture of Claim 40, wherein greater than approximately 90% of the neural cells express tyrosine hydroxylase.
42. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the human cell culture enriched in neural cells of Claim 39.
- 30 43. The method of Claim 42, wherein the neural disease is Parkinson's disease.
44. A method of culturing a human pluripotent cell comprising,
- a) providing a human pluripotent cell culture;

- b) passaging the cell culture using a protease treatment to thereby disperse the cell to an essentially single cell culture; and
  - c) culturing the essentially single cell culture in the presence of a feeder cell, a conditioned medium, or a minimal medium
- 5 to thereby culture the human pluripotent cell.
45. The method of Claim 44, wherein the protease treatment comprises the sequential use of Collagenase and trypsin.
46. The method of Claim 45, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is
- 10 used at a concentration of approximately 0.05% for approximately 30 seconds.
47. The method of Claim 44, wherein the human pluripotent cell is selected from the group consisting of a human embryonic stem cell, a human inner cell mass (ICM)/epiblast cell, a human primitive ectoderm cell, and a human primordial germ cell.
- 15 48. The method of Claim 44, wherein the human pluripotent cell is a human embryonic stem cell.
49. The method of Claim 44, wherein the feeder cell is a freshly plated feeder cell.
50. The method of Claim 49, wherein the feeder cell is a mouse embryonic fibroblast.
- 20 51. The method of Claim 49, wherein the feeder cell has been plated for less than 10 hours.
52. The method of Claim 49, wherein the feeder cell has been plated for less than 6 hours.
53. The method of Claim 49, wherein the feeder cell has been plated for less than 2
- 25 hours.
54. A human pluripotent cell culture produced by the method of Claim 44, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
55. The human pluripotent cell culture of Claim 54, wherein a majority of the cells
- 30 of the culture have an abnormal karyotype.
56. The cell culture of Claim 55, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
57. The cell culture of Claim 56, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.

58. The cell culture of Claim 55, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
59. The cell culture of Claim 58, wherein at least one of the more than one autosomal chromosomes is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
60. The cell culture of Claim 55, wherein the abnormal karyotype comprises an additional sex chromosome.
61. The cell culture of Claim 60, wherein the karyotype comprises two X chromosomes and one Y chromosome.
62. A method of producing a human cell culture enriched in neural cells comprising,
- a) providing a human pluripotent cell culture;
  - b) passaging the cell culture using a protease treatment to thereby disperse the cell culture to an essentially single cell culture;
  - c) culturing the essentially single cell culture in the presence of a feeder cell, a conditioned medium, or a minimal medium; and
  - d) forming an embryoid body comprising the essentially single cell culture by culturing the cell culture with an essentially serum free medium,
- to thereby produce the human cell culture enriched in neural cells.
63. The method of Claim 62, wherein protease treatment comprises the sequential use of Collagenase and trypsin.
64. The method of Claim 63, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.
65. The method of Claim 62, wherein the essentially serum free medium is a MEDII conditioned medium.
66. The method of Claim 65, wherein the MEDII conditioned medium is a Hep G2 conditioned medium.
67. The method of Claim 65, wherein the MEDII conditioned medium comprises one or more proline residues or a polypeptide containing proline residues.
68. The method of Claim 67, wherein the MEDII conditioned medium comprises proline at a concentration of approximately 50  $\mu$ M.

69. The method of Claim 62, wherein the feeder cell is a freshly plated feeder cell.
70. The method of Claim 69, wherein the feeder cell is a mouse embryonic fibroblast.
71. The method of Claim 69, wherein the feeder cell has been plated for less than  
5 10 hours.
72. The method of Claim 69, wherein the feeder cell has been plated for less than 6 hours.
73. The method of Claim 69, wherein the feeder cell has been plated for less than 2 hours.
- 10 74. The method of Claim 62, wherein the minimal medium comprises one or more proline residues, or a polypeptide containing proline residues.
75. The method of Claim 74, wherein the minimal medium comprises proline at a concentration from approximately 50  $\mu$ M to approximately 250  $\mu$ M.
76. The method of Claim 62, wherein the minimal medium is essentially proline  
15 free.
77. The method of Claim 62, wherein the minimal medium is essentially FGF free.
78. The method of Claim 62, wherein the minimal medium is essentially MEDII free.
79. The method of Claim 62, wherein the human pluripotent cell culture is selected  
20 from the group consisting of a human embryonic stem cell culture, a human inner cell mass (ICM)/epiblast cell culture, a human primitive ectoderm cell culture, and a human primordial germ cell culture.
80. The method of Claim 62, wherein the human pluripotent cell culture is a human embryonic stem cell culture.
- 25 81. A human cell culture enriched in neural cells produced by the method of Claim 62.
82. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the neural cell of Claim 81.
- 30 83. The method of Claim 82, wherein the neural disease is Parkinson's disease.
84. The human cell culture of Claim 81, wherein greater than approximately 80% of the human cell culture comprises neural cells.

85. The human cell culture of Claim 84, wherein greater than approximately 90% of the neural cells express tyrosine hydroxylase.
86. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the human cell culture enriched in neural cells of Claim 81.
- 5 87. The method of Claim 86, wherein the neural disease is Parkinson's disease.